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PHYSIOLOGY OF EX VITRO PINEAPPLE PLANTS (ANANNAS COMOSUS L. (MERR) BRS IMPERIAL) SUBMITTED UNDER DIFFERENT LEVELS OF NUTRIENTS.

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ABSTRACT

Pineapple (*Ananas comosus* L. Merr) a specie of Bromeliaceae Family is one of the most commercially important fruit. In Amazon, specifically on Tapajós Basin is largely cultivated in family agricultural contexts with high productivities. However, in the last years, the fusariosis on Pérola cultivate culminated to reduce local productivity of pineapple, associated with water stress. In this context, a new cultivar of pineapple, *BRS IMPERIAL* (EMBRAPA) that is resistant to fusariosis was introduced in a region. The objective of this work was to evaluate the physiological behavior of ex vitro pineapple *BRS IMPERIAL* throw nine variables, 1. Photosynthetic rate (A), 2. Stomatal conductance (GS); 3. Evapotranspiratory rate (E); 4. Leaf Temperature (° C), 5. Total dry matter (MS); 6. Leaf Dry Matter (PA); 7. Root dry matter (SR) 8. PA / SR ratio; 9. Specific Leaf Area (AFE) under different levels of nitrogen, phosphorus and potassium (NPK). The Multivariate Analysis results showed a high similarity (P*hi*= 88%) between the physiological behavior based on the parameters of photosynthetic rate, stomatal conductance and evapotranspiration in pineapple ex vitro leaves when submitted to three different cultivation substrates as demonstrated by the high maximum coefficient ($p < 0.0001$). The leaf temperatures were lower (38.6 ° C) in control treatment (without NPK), compared to high leaf temperatures (46.0 ° C) observed in NPK treatments, suggested that the metabolism CAM must acting on plants with significative levels of NPK. High plasticity on physiological behavior on ex vitro pineapple BRS IMPERIAL was observed and suggested the turn C3/CAM metabolism in young plants.

KEYWORDS: photosynthesis, CAM metabolism, specific leaf area, evapotranspiration, agriculture

INTRODUCTION:

Pineapple (*Ananas comosus* L. Merr) a specie of Bromeliaceae Family differs from most other commercial crops cause a photosynthetic adaptation (crassulacean acid metabolism (CAM)) that facilitates the uptake of CO_2 at night and improves its water-use efficiency under dry conditions. The center of pineapple origin is northern South America, while Brazil, Thailand and Philippines are center of production. The *Ananas* could be consider a plant with high resilience, because your double physiological behavior, in the young phase, it is C3 metabolism, but on adult phase it is CAM metabolism, that is an advantage on actual climate change. This ecophysiological behavior signalizes that *Ananas* as CAM plant has many physiological advantages from others crops. Even though in last decade at Amazon, severe droughts have occurred more frequently in agricultural ecosystems in Tapajos region, which declined the pineapple productivity as occurred at Mojuí dos Campos (IBGE-2016). In accordance with Couto et al (2016), studying ex vitro plants of Pineapple (*Ananas comosus* L.) about net photosynthesis rate, growth and photochemical efficiency observed that the greater growth (canopy diameter and height, shoot dry mass, total dry mass and leaf area) was on 'IAC Fantástico' associated with CAM metabolism. In contrast, 'Vitória' exhibited C_3 metabolism and reduced growth at 60 and 75 DA. The authors concluded that the stress imposed by the environmental conditions induced CAM metabolism in 'IAC Fantástico' and increased growth gain characteristics at 60–70 DA. These results are in accordance with Aragon et al (2012), who studied ex vitro pineapple plants, divided in two sets and subjected to C3 and CAM-inducing environmental conditions, determined by light intensity and relative humidity, respectively, 40 $\mu mol\ m^{-2}\ s^{-1}$/85% and 260 $\mu mol\ m^{-2}\ s^{-1}$/50%. The results were that the stress imposed by the environmental conditions switched pineapple plants from C3 to CAM behaviour. The authors concluded also that C3-induced pineapple plants showed substandard growth parameters and morphological leaf characteristics but a better rooting process and a higher ABA production. It's very clear the physiological plasticity in *Ananas.* So, the physiological metabolism is completely different in function of cultivar of *Ananas,* under stress environmental conditions. Second Caar (2012), revewing the water relations in pineapple observe that it is mainly a rainfed crop, it is widely irrigated. Drip irrigation is successfully used where the water supply is restricted. But unfortunately, in the Amazonian agroecosystems the most of pineapple systems production are without irrigation. This scenar configures a technological gap for bring news varieties of *Ananas* as *BRS IMPERIAL* cultivar, because it is sensible for water stress, but with better characteristics than Pérola cultivar (mainly cultivated), as fusarium resistance and leaf without thorns. In accordance with Azevedo et al (2016) in studies to determine the water requirements of the pineapple crop (*Ananas comosus* L.), cv. "Pérola" grown in the Coastal table lands of Paraíba state, Brazil, using a sprinkler irrigation system, verified that the mean daily crop evapotranspiration was too variable with values decreasing from Evapotranspiration $(ET)_c$ = 4.6 mm day^{-1} in the vegetative growth to 3.5 mm day^{-1} in the fruits harvesting phenological stage. The authors complemented that, ET_c was lower in the beginning of the vegetative growth and fruits harvest and higher in the middle of the productive cycle. These results are determinants for the increase or not the productive of *Ananas* comosus. In the western region of Pará, it is common to use the PEROLA cultivar, because it is more resistant to water shortage, but is susceptible to fusariosis. In this context, it is necessary to introduce new resistances varieties, as BRS IMPERIAL (EMBRAPA, 2004), which is resistant to fusariosis, but more susceptible to water stress. Second Moreira et al (2016) when studying the inoculation with symbiotic fungi, Arbuscular mycorrhizal fungi (AMF) and/or *Piriformospora Indica* on the growth, nutrient absorption, and induction of antioxidant enzyme activities in plantlets of pineapple 'BRS IMPERIAL' (fusariosis-resistant) and 'Pérola' (fusariosis-susceptible) in the presence of *Fusarium subglutinans* f. sp. *Ananas* the results showed that inoculated plantlets with mixture of all the fungi also exhibited a better growth and nutrient absorption, and generally, the 'BRS IMPERIAL' responded better than 'Pérola', suggesting that this practice could be giving more resistances plants for field than the others don't treated. The general objective of this research was to characterize aspects of physiological behavior of ex vitro Abacaxi (*Anannas comosus cultivar BRS IMPERIAL*) seedlings produced under different levels of nitrogen, phosphorus and potassium.

MATERIAL AND METHODS:

The ex vitro of pineapple cv. BRS IMPERIAL multiplied experimentally at the Laboratory of Studies of Amazonian Ecosystems, Federal University of West Pará. At the multiplication stage, four subcultures were performed at 45-day intervals using the Murashige & Skoog culture substrate (1962), plus 0.05 mg L^{-1} benzylaminopurine (BAP) and 0.02 mg L^{-1}-naphthalene acetic acid. Subsequently, the material was subculture in rooting substrate (MS without plant regulators) and seedlings washed for agar removal. Then, the seedlings were transplanted into tubes containing coconut fiber, with PAR OF 1.800 $\mu mol / m^2 / s$ 60% of U.R. The treatments of fertilization were: T1: control (without fertilization); T2: 1,4g NPK (14-16-18)/L substrate (coconut fiber) + 1,4g NPK (06-19-10)/L substrate; T3: 1,4g NPK (14-16-18)/ L substrate; T4: 1,4g NPK (06/19/10)/ L substrate; T5: 0,7g NPK (14-16-18)/L substrate + 0,7g NPK (06-19-10) L substrate; T6: 0,7g NPK (14-16-18) L substrate; T7: 0,7g NPK (06-19-10) L substrate. The plant material remained 60 days in green house with 70% shading. Irrigation was done by micro spray twice a day for 10 minutes. The experiment was a randomized block with 20 replicates / treatment. The variables studied in the ex vitro plants of *Ananas comosus* var *BRS IMPERIAL* were: 1. Photosynthetic rate (A) in $\mu mol\ CO_2 / m^2 / s$, 2. Stomatal conductance (GS) in mmol $H_2O / m^2 / s$; .3. Evapotranspiratory rate (E) in mmol of $H_2O / m_2 / s$; 4. Leaf Temperature (°C), 5. Total dry matter (gr); 6. Dry matter of the aerial part (PA); 7. Root dry matter (SR) 8. PA / SR ratio; 9. Specific Leaf Area (AFE) in cm^2 / gr. The first four variables were measured by an IRGA (LCPRO +, ADC SCIENTIFIC). From the fifth to the eighth variable, an air forced convection oven at 100 ° C was used to dry leaves and roots until constant weight. of *Ananas comosus* cultivar *BRS IMPERIAL*. And the ninth variable was calculated using a Delta Digital WD3 WINDIAS Digital Leaf Area Analyzer. The analysis statistical was Analysis of Variance, Multivariate Analysis (Bartllet Test) and Regressions Analysis. The Statistical Program used was BioEstat 5.3 (Ayres et al, 2007).

RESULTS AND DISCUSSIONS:

The results showed a high similarity between the physiological behavior based on the parameters of photosynthetic rate, stomatal conductance and evapotranspiration in pineapple ex vitro leaves (*Anannas comosus* cultivar *BRS IMPERIAL*) when submitted to three different cultivation substrates as demonstrated by the high maximum coefficient (p <0.0001). These results of this multivariate analysis showed a certain physiological response homogeneity of the pineapple ex vitro plants, regardless of the NPK dosage.

Table 1: Multivariate analysis, through the Bartlett test, for comparison of three multivariate samples, corresponding to three different substrate dosages for ex vitro pineapple (*Ananas comosus cultivar BRS IMPERIAL*), treatment 1 = control, treatment 5 = 0,7g NPK 14-16-18/l + 0,7g NPK 19-6-10/l and treatment 6 = 1,4g NPK 14-16-18/l using a set of three variables; cultivar1 = photosynthesis (A) in µmol CO_2 / m^2 / s, cultivar2 = Stomatal conductance (GS) in mmol of H_2O / m2 / s; cultivar3 = Evapotranspiration (E) mmol H_2O / m^2 / s.

Variable (Var)	Cultivar 1 (photosynthesis=A)	Cultivar 2 (Stomatal conductance= GS)	Cultivar 3 (Evapotranspiration= E)
Matrix T			
	79988.6234	6.1131	-5292.8469
	6.1131	0.0027	-1.4341
	-5292.8469	-1.4341	6749.8276
Matrix W			
	63553.3944	4.9418	65.0406
	4.9418	0.0024	0.0639
	65.0406	0.0639	17.0974
Phi =	88.3339	---	---
(p) =	< 0.0001	---	---

Thus, the higher NPK dosages (culture substrates 2 and 3) supplied to the ex vitro pineapple cultivar *BRS IMPERIAL*, which would promote better mineral nutrition of these plants specifically in relation to the macronutrients nitrogen, phosphorus and potassium, associated to protein synthesis, formation of ATPs and osmotic regulation, did not interfere in the processes of gas exchange like photosynthesis and evapotranspiration, which suggests that the management of such ex vitro seedlings of Ananas comosus cultivar BRS IMPERIAL, at this early stage of life, would not need significant increases in NPK. This context shows that the physiological behavior very similar to some aspects related to the gas exchange in leaf tissues of ex vitro pineapple cultivar BRS IMPERIAL although cultivated with different dosages of NPK is due to the strong influence of the metabolism of the Crassulacean, where the pineapple is one of these species, keeping throughout the day the stomata closed and at night, open. However, when we added the temperature variable to the set of gaseous exchange variables, the physiological behavior among the ex vitro pineapple cultivar BRS IMPERIAL differed significantly at the 1% probability level between the control (without NPK) substrate and the others with NPK, with very low coefficient of similarity Phi = 31.71, according to Table 2. Thus, a reduction of similarity in the physiological behavior of about 57% was observed in relation to the comparison made with only the gas exchange variables. The lowest leaf temperature was observed in treatment without NPK addition (38.68 °C) and the highest averages in the treatments with addition of NPK (46.06 °C and 45.56 °C) respectively treatment 2 and 3 according to figure 2. Such data suggest that less favorable thermal environments in leaf tissues of pineapple ex vitro cultivar BRS IMPERIAL were found on substrates with addition of 0,7g NPK 14-16-18/l + 0,7g NPK 19-6-10/l or 1,4g NPK 14-16-18/l. Considering that temperatures above 30 ° C make the enzymatic activity of most enzymes unfeasible, then it is necessary to adequately adjust the NPK dosages in the initial stages of growth of pineapple cultivar *BRS IMPERIAL*, where mineral nutrition and leaf temperature can act in a favorable way to the development of plants.

Table 2: Multivariate analysis (Bartlett's test) for comparison of three multivariate samples (substrate dosages) for pineapple ex vitro (Ananas comosus cultivar BRS IMPERIAL), treatment 1 = control, treatment 5 = 0,7 g / l of NPK 14-16-18 + 0,7g / L of NPK 19-6-10 and treatment 6 = 1,4g / L of NPK 14-16-18, using a set of four variables; cultivar1 = photosynthesis (A) in µmol CO_2 / m^2 / s, cultivar2 = Stomatal conductance (GS) in mmol of H_2O / m2 / s; cultivar3 = Evapotranspiration (E) mmol H_2O / m^2 / s.; 4. Temperature (°C).

Variable	Cultivar 1 (photosynthesis =A)	Cultivar 2 (Stomatal conductance = GS)	Cultivar 3 (Evapotranspiration=E)	Cultivar 4 (Leaf temperature= T)
Matrix T				
	79988.6234	6.1131	-25.8195	-1679.4400
	6.1131	0.0027	0.1089	-0.1533
	-25.8195	0.1089	6.1043	6.4257
	-1679.4400	-0.1533	6.4257	196.1733
Matrix W				
	63553.3944	4.9418	34.0438	-10.6422
	4.9418	0.0024	0.0991	-0.0470
	34.0438	0.0991	5.1018	-0.3586
	-10.6422	-0.0470	-0.3586	26.0920
Phi =	31.7105	---	---	---
(p) =	0.0001	---	---	---

Thus, the results of the analysis of variance as shown in Figure 1, characterized a significant difference by 1% of probability between leaf temperature in ex vitro pineapple BRS IMPERIAL in control treatment (without NPK) and treatments 2 and 3 (with NPK), however, there were no differences between 5 and 6 treatments (with different doses of NPK), suggesting that these treatments are some similar.

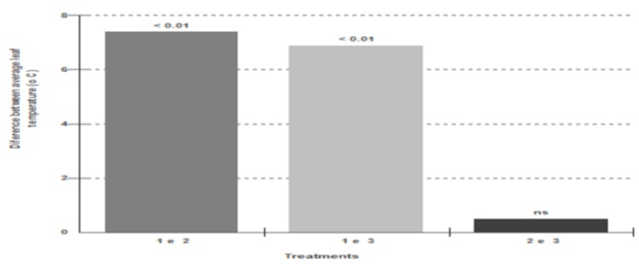

Figure 1: Results of Analysis of variance for leaf temperatures (° C) of pineapple ex vitro (*Ananas comosus* cultivar BRS IMPERIAL) under three treatments (T 1 = control, T 2 = 0,7g NPK 14-16-18/l + 0.7g NPK 19-6-10/l and T 3 = 1,4g NPK (14-16-18)/l UFOPA, Santarém, Para-Brazil.

The leaf temperatures of ex vitro plants of *Ananas comosus* BRS IMPERIAL were lower (38.6 ° C) in the control treatment (without NPK), compared to the high foliar temperatures (46.0 ° C) observed in the NPK treatments, regardless of the dosage according to Figure 2. These data suggest that high temperatures could be related to the high heat rates emitted by the metabolism of ex vitro plants of *Anana comosus* cultivar *BRS IMPERIAL* cultured with such NPK dosages specifically. Such different physiological behavior shows that plants cultivated under control (without NPK) may be C3 metabolism, with open stomates and therefore with higher aeration, thus reducing foliar temperatures in this crassulacean, while the other plants cultivated with NPK would be in metabolism CAM, with closed stomata, lower aeration and higher foliar temperatures, which can be evidenced by the reduction in photosynthesis in these same plants (treatments 2 and 3). It is concluded that, among the 4 physiological variables discussed here, leaf temperature was the best bioindicator, the most sensitive in the identification of distinct physiological behaviors in pineapple cultivar *BRS IMPERIAL*. In accordance with Luttge (2004) the PEPC and malate dehydrogenase reach their optimum at 35 °C and the decarboxylating enzymes, e.g. malic enzyme, at above 53 °C on crassulacean.

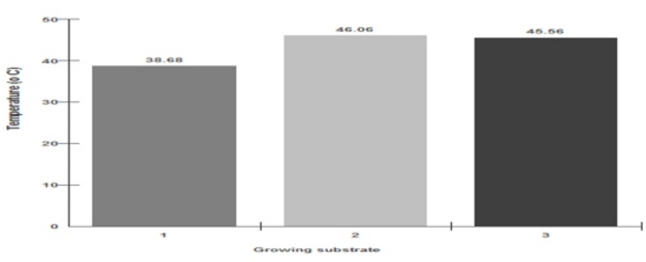

Figure 2: Leaf temperatures of pineapple ex vitro (*Ananas comosus*) cultivar BRS IMPERIAL (T1 = control, T2 = 0,7g NPK 14-16-18/l + 7gL NPK 19-6-10/l and 3 = 1,4g NPK 14-16-18/l, with *p* <0.001 between treatments 1 and 2 and 1 and 3.

The results on photosynthetic rate in leaf tissues of pineapple ex vitro (*Ananas comosus) cultivar BRS IMPERIAL* at 9:00 a.m. - 10:00 a.m. did not show significant differences between fertilization treatments as showed on figure 3, with a tendency of higher photosynthetic rate in the control treatment (120 µmolCO2 / m2 / s). According to Richie and Bunth (2010), studying photosynthesis in plants of Ananas Comosus L. Merr measured by fluorimetry using the pulse amplitude modulation method (PAM), observed that the total CO_2 fixed nocturnal as C_4-dicarboxylic acids by leaves of the Phuket pineapple was only ≈0.14 g C m^{-2} d^{-1} (0.012 mol C m-^{2} d^{-1}). The authors observed that the Titratable acid of leaves depleted about 3 pm (15:00) and shows a classical CAM diurnal. For analogy, the results of this research show low levels of photosynthesis, exactly because of measure time, 9:00 a.m. the most stomatal

were closed, reducing the photosynthetic taxes, independently of growing substrate.

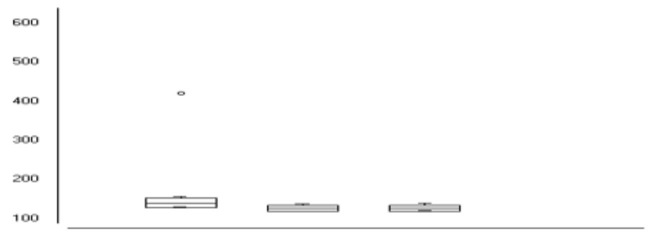

Figure 3: Photosynthesis (µmol CO_2 / m^2 / s) in leaf tissues of pineapple ex vitro (*Ananas comosus) cultivar BRS IMPERIAL* (T 1 = control (without NPK), T 2 = 0,7 g NPK 14-16-18/l + 7 g NPK 19-6 -10/l and T 3 = 14g NPK 14-16-18/l. LEEA-UFOPA-Brazil.

When we related the evapotranspiration data to those of stomatal conductance in leaf tissues of pineapple ex vitro *(Ananas comosus) cultivar BRS IMPERIAL* on treatment with 0,7 g NPK 14-16-18/l + 0,7 g NPK 19-6-10/l the results showed a high fit for the linear model between the two variables with determination coefficient R2 = 99.47% according to Figure 4, such results being significant at the 1% probability level (p = 0.0022). Thus, evapotranspiration increases linearly with the increase in stomatal conductance only for this crop treatment (T2). Thus, the loss of water vapor via stomata contributes greatly to the overall Evapotranspiration process due to the linear behavior between them.

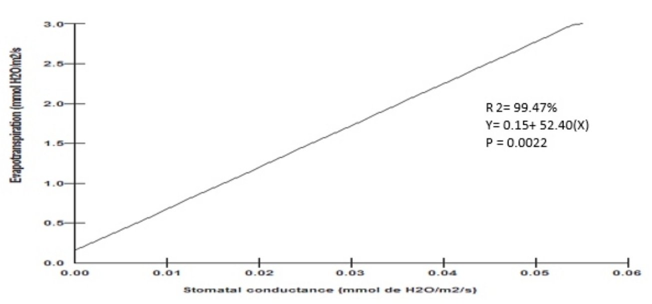

Figure 4: Linear relationship between evapotranspiration and stomatal conductance in leaf tissues of pineapple ex vitro (*Ananas comosus) cultivar BRS IMPERIAL* from 9: 00-10: 00 a.m. in treatment with 0,7 g NPK 14-16-18/l + 0,7 g NPK 19-6-10/l.

Regarding the results of specific leaf area (AFE) of leaves of pineapple seedlings *Ananas comosus cultivar BRS IMPERIAL* submitted to different dosages of NPK, no significant differences were observed at 5% probability level according to figure 5; indicating that, regardless of the NPK increase, this was not enough to cause changes in the leaf area / dry matter ratio, or in other words, there is no difference between the size of the leaf area used in the production of 1 gram of leaf phytomass among the 7 treatments. However, treatment 3 with 1,4g / L of NPK 14-16-18 had the lowest AFE, showing a trend of higher efficiency of these plants in the use of leaf area to produce 1 gram of leaf dry matter. These results are explained by the effect of nitrogen on protein synthesis, phosphorus in phosphorylation processes and the effect of potassium on osmotic regulation, which may be acting to justify, among other factors, the low AFE in this fertilization treatment.

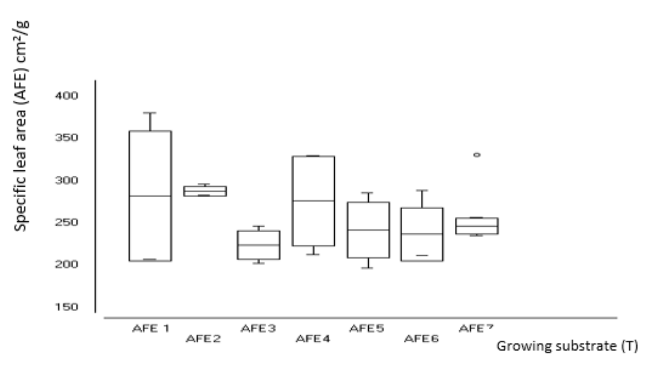

Figure 5: Specific Leaf Areas (AFE), mean (mid-box lines) and standard deviations (upper and lower box lines) of ex vitro plants of *Ananas comosus cultivar BRS IMPERIAL* under following treatments; T1 = control, T2 = 1,4g NPK 14-16-18/l + 1,4g NPK 19-6-10/l, T3 = 1,4g NPK 14-16-18/l, T4 = 1,4g NPK 19- 6-10/l, T5 = 0,7g NPK 14-16-18/l + 0,7g / L NPK 19-6-10, T6 = 0,7g / L NPK 14-16-18 & T7 = 0,7g / L NPK 19- 6-10. * The means did not differ by Test T at 5% probability.

Regarding the leaf dry matter / root dry matter (g / g) ratio of pineapple EX VITRO cultivar *BRS IMPERIAL* showed to be significantly different at the 5% probability level as shown in figure 6. It was observed that the control treatment, where it does not have NPK, obtained the lowest ratio, that is, almost the same proportion of leaf dry matter in relation to root dry matter. However, when added if treatments with NPK identified an increase of these ratios, markedly for treatment 2 with 1,4g / NPK 14-16-18/l + 1,4g NPK 19-6-10/l, indicating that leaf phytomass was much larger than the root, which is a positive factor from the point of view of the vegetative growth of ex vitro cultivar *BRS IMPERIAL* with the possibility of reducing the beginning of flowering period. From the physiological point of view, a mean leaf dry matter / dry matter ratio around 3.0 g / g in ex vitro pineapple seedlings induced by NPK treatments indicates a good carbon fixation and distribution to the aerial part in detriment of the suggesting larger leaf areas for photosynthetic processes. However, from the economic point of view, such fertilizers still must be carefully evaluated.

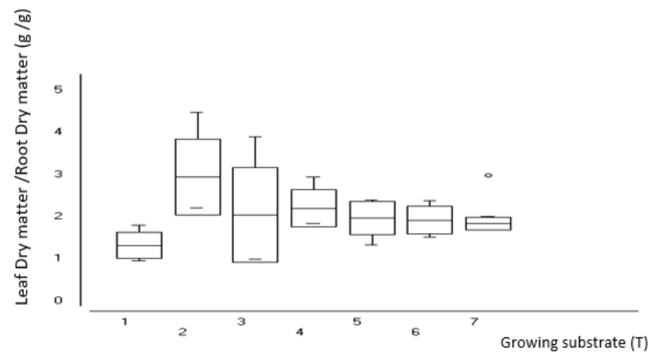

Figure 6: Ratios (Leaf dry matter/root dry matter) of ex vitro pineapple seedlings (*Ananas comosus var BRS IMPERIAL*) under seven different NPK treatments; T1 = control, T2 = 1,4g NPK 14-16-18/l + 1,4g NPK 19-6-10/l, T3 = 1,4g NPK 14 -16-18/l, T4 = 14g NPK 19-6-10/l, T5 = 7g NPK 14-16-18/l + 7g NPK 19-6-10/l, T6 = 7g NPK 14 -16-18/l & T7 = 7g NPK 19-6-10/l. * means differed by Test T, p < 0.05.

According to the Pearson Linear Correlation Matrix (Table 3), the data showed a high positive correlation (r = 0.9081) among the aerial dry matter and root dry matter of pineapple ex vitro cultivar *BRS IMPERIAL*, where the increase of aerial dry matter increases the root dry matter also and can be explained such linear behavior, determined by 82.46% (R2) only for the control treatment, that is, without NPK. This physiological behavior shows the balance between dry matter distribution on aerial and root system on pineapple ex vitro plants, suggesting that the photosynthetic tools throw leaves, and nutrients assimilation tools, thrown roots systems, growth for maximum development of vegetative phase. For the other fertilization treatments in ex vitro pineapple cultivar *BRS IMPERIAL* did not have significant correlations to be explained by linear model, indicating that fertilization with NPK changes the relationship between the variables studied, suggesting that other mathematical models may be explaining such relationships. The high ratio, leaf dry matter/root dry matter, specifically on treatment 2 with high levels of phosphorus promoted a high level of leaf dry matter in these early stages. If this behavior continue throw vegetative cicle until the appearance of leaf *D,* so this could culminate with fruits with high weigh, as well observed Junghans et al (2016) that studied pineapple plants of BRS IMPERIAL observed a hhighly positive and significant correlations between fresh 'D' leaf weight and fruit weight without (r=0.74) and with (r=0.72) crown, as well, a moderate and positive correlation between 'D' leaf length and fruit weight without (r=0.65) and with (r=0.65) crown.

Table 3: Pearson Linear Correlation Matrix between six (6) variables (1 = leaf area, 2 = leaf dry matter, 3 = AFE, 4 = Aerial dry matter; 5 = root dry matter, 6 = leaf dry matter /root dry matter in ex vitro pineapple plants (*Ananas comosus cultivar BRS IMPERIAL*) in T1 = control.

Variables	1 e 2	2 e 4	4 e 5	5 e 6
n (pairs) =	5	5	5	5
r (Pearson) =	0.8320	0.8729	0.9081	-0.8204
R2 =	0.6922	0.7619	0.8246	0.6731
(p) =	0.0805	0.0533	0.0330	0.0888

The results about Regression Analysis shows the two predominant mathematical adjustments between variables, basically exponential and linear behaviors. Among aerial and root dry matter on pineapple *ex vitro* plants (var *BRS IMPERIAL*) the adjustments were exponential as showed in Figures 7,8 and 9 in different treatments of NPK. The dry root matter growths exponentially while increasing leaf dry matter. This physiological behavior signalizes that the increase on leaf dry matter imposes aggressive growth of root matter to support needs of water and nutrients above soil. These results indicate indirectly that phosphorus supply on soil for ex vitro plants of *Ananas comosus cultivar BRS IMPERIAL* it is very important for well development of root systems.

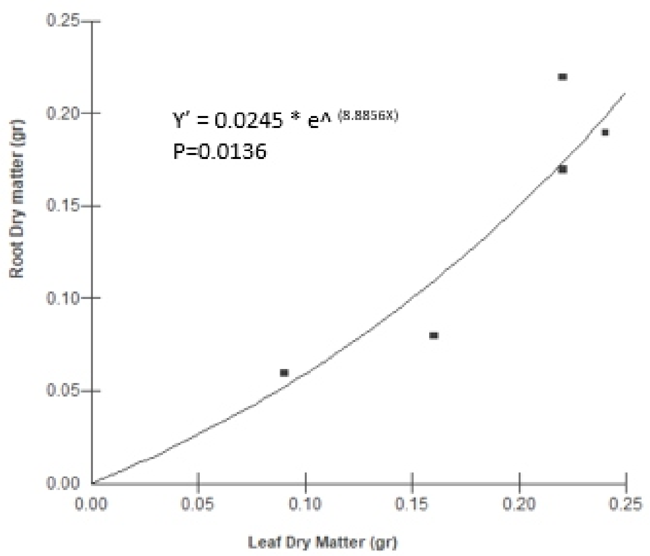

Figure 7: Exponential adjustment between root dry matter (gr) and leaf dry matter (gr) on *ex vitro* plants of *Ananas comosus var. Imperial* under control treatment.

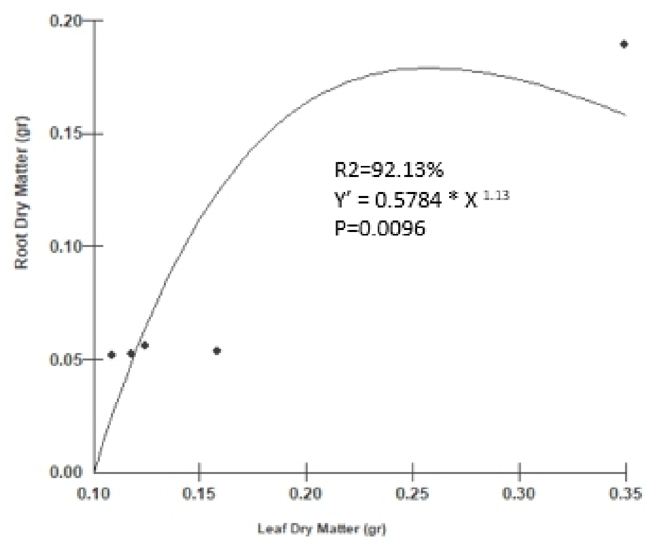

Figure 8: Exponential adjustment between root dry matter (gr) and leaf dry matter (gr) on *ex vitro* plants of *Ananas comosus var. Imperial* under T4 = 1,4g NPK 19-6-10/l.

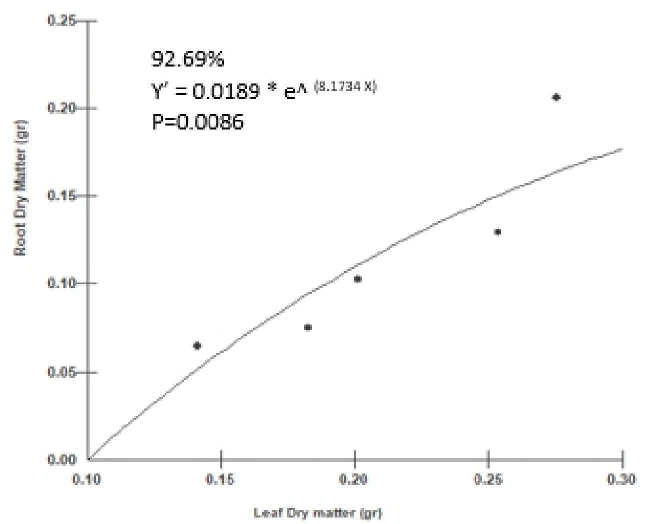

Figure 9: Exponential adjustment between root dry matter (gr) and leaf dry matter (gr) on *ex vitro* plants of *Ananas comosus var. Imperial* under T5=0,7g NPK 14-16-18/l + 0,7g NPK 19-6-10/l

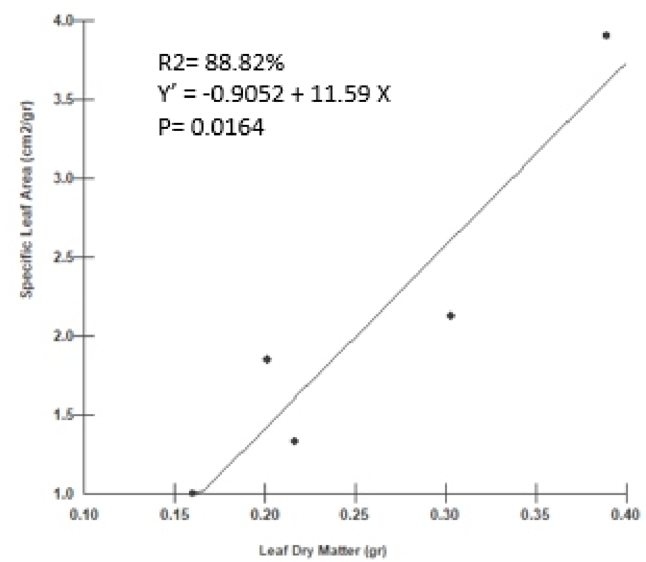

Figure 10: Linear adjustment between Specific Leaf Area (cm^2/gr) and leaf dry matter (gr) on *ex vitro* plants of *Ananas comosus var. Imperial* under T3= 1,4g NPK 14 -16-18/l

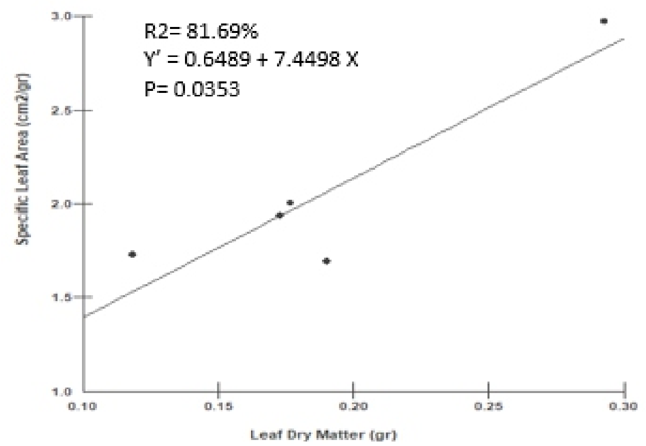

Figure 11: Linear adjustment between Specific Leaf Area (cm^2/gr) and leaf dry matter (gr) on *ex vitro* plants of *Ananas comosus var. Imperial* under T7= 0,7g NPK 19-6-10/l

These results are in accordance with Cruz et al (2015) that evaluated mycorrhizal association, growth and nutrient uptake of 'BRS IMPERIAL' micro propagated pineapple plants inoculated with the fungus *Piriformospora indica* in cultivation with herbicide application, found that 'BRS IMPERIAL' pineapple nursery plants associated with *P. indica* fungus showed higher levels of nutrients and growth, exactly cause the high availability of phosphorus throw fungus to plant. Considering the poor availability of Amazonian soils and the results of this research, it's very recommendable the addiction of high supplies of phosphorus in the early stages of pineapple ex vitro plants of cultivar BRS IMPERIAL. Many studies with pineapple varieties showing the reduced growth of D leaf due to nutritional stress. In accordance with Ramos e Pinho (2009), the deficiencies of N and K reduced fresh and dry weight, length, width and area of 'D' leaves at nine and 12 months after planting; that of Ca reduced fresh weight and 'D' leaf area at 12 months and the deficiencies of P, Mg, S and B did not significantly affect the leaf variables. Second Figures 10th and 11th above, the increase on specific leaf area with increase on leaf dry matter in T3 and T7 treatments represents a low efficiency of these ex vitro plants of pineapple cultivar *BRS IMPERIAL* in using leaf area to convert in weigh of dry matter.

CONCLUSIONS:

Ex vitro plants of pineapple *cultivar BRS IMPERIAL* cultivated under different levels of NPK showed heterogeneity of physiological behavior. Plants without addiction of NPK has more balance between leaf dry matter and root dry matter than plants cultivated under NPK. Leaf Temperature was the best physiological indicator of stress, cause high leaf temperatures on plants under NPK treatments, signalizes the occurrence of CAM metabolism induced for increasing soil osmotic potential. The growth of root system of *ex vitro* plants of pineapple cultivar *BRS IMPERIAL* is strongly induced for an increase of leaf dry matter. The increasing of Specific Leaf Area with increase of leaf dry matter, demonstrates low efficiency of pineapple to use leaf area to convert CO_2 in

carbon mass under high levels of NPK.

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